C1 Cont.

28. An isolated non-toxic, non-toxigenic, non-pathogenic *Fusarium venenatum* host cell, having the identifying characteristics of *Fusarium venenatum* ATCC 20334, comprising a nucleic acid sequence encoding a heterologous protein.

## REMARKS

Claims 23-25 have been canceled. New claims 26-28 have been added and are pending in the present application.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

### I. The Rejection of Claims 23-25 under 35 U.S.C. § 112, First Paragraph

Claims 23-25 stand rejected under 35 U.S.C. § 112, first paragraph, "because the specification, while being enabling for the use of cells deposited at ATCC under deposit number 20334, does not reasonably provide enablement for the use of recombinant host cell having the identifving characteristics of a non-toxic, non-toxigenic, non-pathogenic *Fusarium venenatum* host cell." The Office Action specifically states:

> The specification is silent with respect to what identifying characteristics enable correct identification of *Fusarium venenatum.* Colony morphology of *Fusarium graminearum* on PDA are specifically provided which ultimately are not sufficient for *Fusarium venenatum.* As we are taught in Yoder *et al.* morphological characteristics are best discernible between strains by growth on minimal media. Cultural characteristics recited in the specification most closely resemble *Fusarium graminearum* and not *venenatum.* Required techniques for the proper execution of identifying *Fusarium venenatum* as well as critical primers and unique cultural details are not provided in the specification. Therefore, no disclosure for a critical aspect of the invention, host cells having the identifying characteristics of a non-toxic, non-toxigenic, non-pathogenic *Fusarium venenatum* host cell is provided.

This rejection is respectfully traversed.

The Office Action asserts that the skilled artisan would have to conduct undue experimentation and excessive experimentation in order to practice the claimed invention because the specification is silent with respect to what identifying characteristics enable correct identification of *Fusarium venenatum*. Applicants disagree with this assertion for several reasons.

First, Applicants respectively point out that taxonomic identification is an evolving area of expertise. As methodologies improve, taxonomic identification improves. Taxonomic identification is

handled by experts known in the art. While at the time of filing of the instant application, there were no published reports of *Fusarium venenatum* strains, the state of the art was in place to identify *Fusarium* strains as belonging to the species *venenatum*. H.I. Nirnberg, a world-renowned taxonomist, had submitted a paper on September 8, 1993 for publication in the journal *Mycopathologia*. Please refer to Nirenberg in *Mycopathologia* 129: 131-141, 1995; see Appendix H of Dr. Yoder's Declaration of April 19, 2000. Applicants submit that the state of art in taxonomic classification of *Fusarium venenatum* strains at the time of filing of the application was in place prior to September 8, 1993, as is described in the Nirenberg reference, which was submitted for publication on September 8, 1993. In fact, *Fusarium* ATCC 20334 was submitted blind to the laboratory of Nirenberg for taxonomic classification, after the filing date, which correctly identified the strain as *F. venenatum*. Since the capability to identify a strain as a *Fusarium venenatum* was therefore in existence by a major laboratory involved in fungal taxonomic classification at the time of filing, one of ordinary skill in the art could properly identify a *Fusarium venenatum* strain to determine whether the strain falls within the scope of Applicants' invention.

Second, the Office Action suggests that cultural and/or morphological characteristics are not reliable in identifying strains. Applicants respectfully disagree with the Office Action's assertion that the initial misidentification of the strain designated ATCC 20334 as *F. graminearum* implied that species and strain designations based solely on cultural and/or morphological characteristics are not reliable, consistent or accurate. In taxonomic classification, cultural and/or morphological characteristics are routinely used to determine the classification of a strain. Please refer to Nirenberg in *Mycopathologia* 129: 131-141, 1995, which clearly shows that such characteristics are reliable. As noted above, *Fusarium* strain ATCC 20334 was submitted blind to the laboratory of Nirenberg for taxonomic classification, which correctly identified the strain as *F. venenatum*. Nirenberg's taxonomic identification was performed as described in her paper *Mycopathologia* 129: 131-141, 1995. However, the Office Action asserts that "[a]s we are taught in Yoder *et al.* morphological characteristics are best discernible between strains by growth on minimal media. Cultural characteristics recited in the specification most closely resemble *Fusarium graminearum* and not *venenatum.*" Applicants disagree with the Office Action's contention that "[c]ultural characteristics recited in the specification most closely resemble *Fusarium graminearum* and not *venenatum*" The characteristics described on page 4, line 26 to page 5, line 2, and page 5, line 24, to page 6, line 3, were describing the ATCC 20334 strain, which was really *Fusarium venenatum*, not *Fusarium graminearum*. Moreover, one of ordinary skill in the art would recognize the need to submit such a strain with such features and characteristics to an expert *Fusarium* taxonomist, such as Nirenberg, to confirm the strain is a *Fusarium venenatum* strain.

Third, the inherent characteristics and features of the ATCC 20334 strain did not change, just the species name. These inherent characteristics and features existed at the time of filing of the application and have not changed. Even though Nirenberg's paper did not publish until after the filing date, this should not limit the scope of protection of Applicants' invention because the knowledge existed in the art at the time of filing, as noted above. Supplemental methods in the art of taxonomy which were developed after the filing date, such as RAPD PCR, to confirm the identification of strains do not negate the reality that the identifying characteristics of *Fusarium* strain ATCC 20334 existed at the time of filing and have not changed. The Office Action indicates that Applicants' have not disclosed the identifying characteristics of a *Fusarium venenatum* strain. Applicants assert that a taxonomist skilled in the art could identify these characteristics as Nirenberg did prior to the date of September 8, 1993. Thus, ordinary skill in the art existed at the time of filing to accomplish the proper identification.

Fourth, the Office Action asserts that it would require undue experimentation to determine whether a *Fusarium venenatum* strain is non-toxic, non-toxigenic, and non-pathogenic. Applicants describe on page 5, lines 3-23, of the specification methods well known in the art for determining whether a strain is non-toxic, non-toxigenic, and non-pathogenic. One of ordinary skill in the art would have no difficulty in determining whether a *Fusarium venenatum* strain is non-toxic, non-toxigenic, and non-pathogenic.

Sixth, limiting the claims to *Fusarium* strain ATCC 20334 would not adequately protect the inventors. Based on the teachings of the present application, one skilled in the art could find another non-toxic, non-toxigenic, and non-pathogenic *Fusarium venenatum* strain having essentially the same properties as that of *Fusarium venenatum* strain ATCC 20334 and thereby circumvent the literal scope of Applicants' patent rights. For example, O'Donnell *et al.* in *Fungal Genetics and Biology* 23: 57-67, 1998 (see Appendix I of Dr. Yoder's Declaration of April 19, 2000), describe such a situation. O'Donnell *et al.* disclose in Table 3 on page 60 that *F. venenatum* NRRL 26139=BBA 64537 produces no toxins (see page 58 of reference).

Applicants assert, therefore, the specification, while being enabling for the use of cells deposited as ATCC 20334, also provides enablement for the use of recombinant host cells having the identifying characteristics of the non-toxic, non-toxigenic, non-pathogenic *Fusarium venenatum* host cell of ATCC 20334.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

## II. The Rejection of Claims 23-25 under 35 U.S.C. § 112, First Paragraph

Claims 23-25 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Office Action states:

> The written description requirement for genus claims may be satisfied through sufficient description of a relevant representative number of species by actual reduction to practice or by disclosure of relevant identifying characteristics such as structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure or by a combination of such identifying characteristics sufficient to show applicants were in possession of the claimed genus. In the instant case, applicants present as relevant identifying characteristics, colony morphology of (ATCC 20334) *Fusarium graminearum* on PDA. However, this characteristic is not sufficient for *Fusarium venenatum.*

This rejection is respectfully traversed.

For the same reasons explained in section I (see above) and further reasons described below, Applicants assert that the subject matter is described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicants preliminarily note that *Fusarium venenatum* strain ATCC 20334 has been deposited and is publicly available (see Section III below).

The Office Action contends that Applicants' disclosed examples for ATCC 20334 are not a representative number of species to show Applicants were in possession of the claimed genus. Applicants disagree with this contention because Applicants are not claiming the genus *Fusarium*, but rather a very specific species within the Section Discolor, *i.e.*, *Fusarium venenatum*. Moreover, Applicants further specify that the *Fusarium venenatum* must have the identifying characteristics of *Fusarium venenatum* strain ATCC 20334 and must be non-toxic, non-toxigenic, non-pathogenic. Applicants submit that the information disclosed in the specification combined with the knowledge in the art provides sufficient guidance to one of ordinary skill in the art to taxonically identify a non-toxic, non-toxigenic, and non-pathogenic *Fusarium venenatum* strain having the identifying properties as that of *Fusarium venenatum* strain ATCC 20334. Thus, there is sufficient written description in the specification to inform the skilled artisan that Applicants were in possession of the claimed subject matter at the time the application was filed.

Limiting the claims solely to *Fusarium* strain ATCC 20334 would not adequately protect the inventors. Based on the teachings of the present application, one skilled in the art could find another

non-toxic, non-toxigenic, and non-pathogenic *Fusarium venenatum* strain having essentially the same properties as that of *Fusarium venenatum* strain ATCC 20334 and thereby circumvent the literal scope of Applicants' patent rights. For example, as noted in Section I, O'Donnell *et al.* in *Fungal Genetics and Biology* 23: 57-67, 1998 (see Appendix I of Dr. Yoder's Declaration of April 19, 2000), describe such a situation.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

## III. Deposit of *Fusarium* ATCC 20334

The Office Action states that the *Fusarium venenatum* of the invention was apparently deposited by others and since its availability cannot be ensured, Applicants must deposit the specific *Fusarium venenatum* recited in the claims to satisfy a deposit requirement under 37 CFR 1.801-1.809. This request is respectfully traversed.

Under 37 C.F.R. 1.802(b), "[b]iological material need not be deposited, *inter alia*, if it is known and readily available to the public...." When the Patent Office adopted the rules on the deposit of biological materials, it issued comments on interpreting and applying the rules. See 54 FR 34880. The comments regarding the terms "known and readily available" in 37 C.F.R. 1.802(b) are set forth at page 23 of 54 FR 34880 (a copy thereof is enclosed herewith) as follows:

> Even where access to biological material is required to satisfy these statutory requirements, a deposit may not be necessary if access sufficient to satisfy these requirements is otherwise available.
>
> For example, applicant could show that the biological material is known and readily available to the public. The concepts of "known and readily available" are considered to reflect a level of public accessibility to a necessary component of an invention disclosure that is consistent with an ability to make and use the invention. To avoid the need for a deposit on this basis, the biological material must be both known and readily available - neither concept alone is sufficient....
>
> By showing that a biological material is known and readily available or by making a deposit in accordance with these rules, applicant does not guarantee that such biological material will be available forever. Public access during the term of the patent may affect the enforceability of the patent. Although there is a public interest in the availability of the deposited material during and after the period of enforceability of the patent, the examiner need not be unduly concerned about continued access to the public. Unless there is a reasonable basis to believe that the biological material will cease to be available during the life of the patent, the examiner should accept current availability as satisfying the requirement. The incentives

> provided by the patent system should not be constrained by the mere possibility that a disclosure that was once enabling would become non-enabling over a period of time through no fault of the patentee. *In re Metcalfe*, 410 F.2d 1378, 161 USPQ 789 (CCPA 1969) (emphasis added).

Applicants submit that the microorganism recited in the claims is "known and readily available" and, therefore, Applicants do not have to provide the assurances requested in the Office Action.

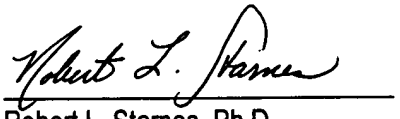
Applicants have enclosed a copy of page 178 of the ATCC catalogue for 1991 which lists *Fusarium* ATCC 20334. Also attached are pages from ATCC's website (www.atc.org) that disclose the strain is commercially available and may be purchased. Clearly, these publications establish that this strain was known prior to Applicants' filing date. Applicants note that they obtained the strain from this depository.

For the foregoing reason, Applicants submit that this rejection under 35 U.S.C. § 112 has been overcome and respectfully request reconsideration and withdrawal of the rejection.

## IV. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,



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*Mycopathologia* **129**: 131–141, 1995.


# Morphological differentiation of *Fusarium sambucinum* Fuckel sensu stricto, *F. torulosum* (Berk. & Curt.) Nirenberg comb. nov. and *F. venenatum* Nirenberg sp. nov.

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**Abstract**

Within *Fusarium sambucinum* Fuckel sensu lato three species were differentiated: *F. sambucinum* Fuckel s. str., *F. torulosum* (Berk. et Curt.) Nirenberg comb. nov. and *F. venenatum* Nirenberg sp. nov. They are described and illustrated in detail.

**Key words:** Cultural characteristics, Fungi imperfecti, *Fusarium sambucinum*, *Fusarium torulosum*, *Fusarium venenatum*, Morphology, Taxonomy

## Introduction

*Fusarium sambucinum* Fuckel sensu lato is found quite often in the temperate zone and in the subtropics. It occurs in soil and on many plants. It is well known to plant pathologists for producing a dry rot on potato tubers in the field and in storage. For further details see Wollenweber [1], Domsch et al. [2], and Gerlach and Nirenberg [3]. This fungus also produces toxic secondary metabolites [4] such as diacetoxyscirpenol.

*F. roseum* Link is the type species of the genus *Fusarium*. Since the epithet *roseum* was used by Snyder and Hansen [5] in a completely different sense, *F. sambucinum* Fuckel being a synonym, replaced it. Therefore *F. sambucinum* is of special significance for *Fusarium* taxonomists. Wollenweber and Reinking [6] placed it in the section *Discolor*.

The present taxonomic views of *Fusarium sambucinum* Fuckel s. l. are shown in Table 1: Wollenweber and Reinking [6] differentiated *Fusarium sambucinum* Fuckel, *F. sambucinum* Fuckel var. *minus* Wollenw. and five formae of which four were based solely on colour differences. Formae 1, 4 and 5 formed more slender sporodochial conidia than the basic species, whereas in formae 2 and 6 the width of the conidia did not differ from the latter. They combined forma 3 which had been described by Wollenweber with *F. culmorum*. They recognized *F. trichothecioides* Wollenw. as a seperate species.

Booth [7] accepted the varieties *F. sambucinum* var. *sambucinum* and *F. sambucinum* var. *coeruleum* as well as *F. sulphureum* Schlecht. and *F. trichothecioides*. *F. sambucinum* represented the red cultures on rich media like potato sucrose agar (PSA), *F. sulphureum* the yellowish and *F. trichothecioides* the rose coloured, pionnotal ones. He also thought that *F. sarcochroum* (Desm.) Sacc. was identical with *F. sambucinum* and *F. bactridioides* Wollenw. with *F. trichothecioides*.

Gerlach & Nirenberg [3] considered *F. trichothecioides* synonymous with *F. sulphureum*. Like Booth they accepted the two varieties *F. sambucinum* var. *sambucinum* and *F. sambucinum* var. *coeruleum*. Their concept of the variety *coeruleum*, like Booth's, was not the same as Wollenweber's and Reiking's. They described the strains of this variety (=f. 1) as having more slender conidia than those of the variety *sambucinum*, whereas according to Booth [7] and Gerlach and Nirenberg [3] the conidia are wider. They recognized *F. bactridioides* and *F. sarcochroum* as good species.

Nelson et al. [8] combined *F. trichothecioides*, *F. bactridioides*, *F. sulphureum*, *F. sambucinum* var. *minus* and *F. sambucinum* var. *coeruleum* with *F. sambucinum* without mentioning *F. sarcochroum*.

*Table 1.* Concept of *Fusarium sambucinum* Fuckel sensu lato by various authors

| Wollenweber & Reinking 1935 [6] | Booth 1971 [7] | Gerlach & Nirenberg 1982 [3] | Nelson et al. 1983 [8] | Nirenberg (present concept) |
|---|---|---|---|---|
| *F. sambucinum* Fuckel | *F. sambucinum* | *F. sambucinum* (all red strains)! | *F. sambucinum* | *F. sambucinum* |
| *F. sambucinum* f. 1 Wollenw. | ! *F. sambucinum* var. *coeruleum*[b] | ! *F. sambucinum* var. *coeruleum* | *F. sambucinum* | *F. torulosum* (Berk. et Curt.) comb. nov. |
| =*F. sambucinum* var. *coeruleum* Wollenw. | | | | |
| =*Fusarium torulosum* Berk. et Curt. | | | | |
| ? *F. culmorum* (W.G. Sm.) Sacc. var. *cerealis* (Cook) Wollenw. pr. p.[a] | | | ?*F. culmorum* | *F. venenatum* sp. nov. |
| *F. sambucinum* f. 2 Wollenw. | *F. sambucinum* var. *coeruleum*![c] | *F. sambucinum* | *F. sambucinum* | *F. sambucinum* |
| *F. sambucinum* f. 4 Wollenw. | ? | *F. sambucinum*! | *F. sambucinum* | *F. torulosum* |
| *F. sambucinum* f. 5 Wollenw. | ? | *F. sambucinum* ! | *F. sambucinum* | *F. torulosum* |
| *F. sambucinum* f. 6 Wollenw. | *F. sulphureum* | *F. sulphureum* | *F. sambucinum* | *F. sambucinum* |
| *F. sambucinum* var. *minus* Wollenw. | *F. sambucinum* | ? *F. sambucinum* | *F. sambucinum* | ? *F. sambucinum* / |
| *F. trichothecioides* Wollenw. | *F. trichothecioides* | ? *F. sulphureum* | *F. sambucinum* | *F. sambucinum* |
| *F. bactridioides* Wollenw. | *F. trichothecioides* | *F. bactridioides*?[d] | *F. sambucinum* | *F. bactridioides* ?- |
| *F. sarcochroum* (Desm.) Sacc. | *F. sambucinum* | *F. sarcochroum* | ? | *F. sarcochroum* ? |

[a]? In front of the taxon = author is not sure about synonymity.

[b]! In front of the taxon = taxon does not correspond with the original description.

[c]! After the taxon = author made an error by placing his concept of the taxon synonymous with that of Wollenweber & Reinking 1935 [6].

[c]? After the taxon = concept may be doubtful.

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The collaborators of the EFS-project intended to reduce these different views on *Fusarium sambucinum* s. 1. They wanted to offer an acceptable concept by conducting as many morphological, physiological, genetical, and biological investigations as possible with relevant strains. The results of one of these investigations, the morphological characterisation of the three species, is presented here.

## Material and methods

Strains of *F. sambucinum* were requested from collaborators of the European *F. sambucinum* (EFS) project and between October 1987 and December 1988 about 140 *Fusarium sambucinum* s. 1. strains were sent to me at the Institute of Microbiology in Berlin-Dahlem. Most of them were isolated from potato tubers in Europe. I typified them according to my previously acquired taxonomic knowledge of *Fusarium sambucinum* s. 1. and arranged them in three different groups (Table 2).

Of the 140 strains, 41 were chosen for detailed study in the EFS-project bearing in mind their association to the group, their host plant, their country of origin, their cultural state (wild type, degenerated culture), and their colour on wort agar or PDA.

Fourteen strains were chosen from group I, eight from group II, six from group III. Five were placed into group IV, representing strains that could not be typified properly, but whose conidia showed certain similarities with those of group I–III. The exholotype of *F. bactridioides* represented group V, one strain of *F. sambucinum* isolated from the teleomorph *Gibberella pseudopulicaris* Wollenw. by E. Müller, represented group VI. Both cultures were incorporated into the project because of Booth's concept. Group VII consisted of two isolates of the teleomorph *Gibberella pulicaris* (Fr. ex Fr.) Sacc. One strain for each of the four species were meant to serve as some kind of control: *F. culmorum* (W. G. Smith) Sacc., *F. cerealis* (Cooke) Sacc. (=*F. crookwellense* Burgess, Nelson et Toussoun) and *F. compactum* (Wollenw.) Gordon were chosen because of the similarities of their conidia with those of group III, and *F. lateritum* Nees because it was placed by Wollenweber and Reinking along with *F. sarcochroum* into the section *Lateritum*. Another reason was a certain similarity between the cultural charcteristics of *F. lateritium* and group II on wort agar.

The 41 *Fusarium* cultures (Table 2) were grown on a synthetic medium with a low nutrient content (SNA) [9] in the laboratory at ca. 20–22 °C and natural day-night-rhythmn, in the dark (D) and under constant black light illumination (DL) at 20 °C for 10 to 28 days. The morphological characteristics were photographed. For the characterisation of the three species within *F. sambucinum* s. 1. (identical with group I–III) measurements were taken of four representative cultures of each group. Their means including standard deviations are given in the description as well as the extremes in parentheses. Since the strains are cultivated under standardised conditions [9] the variation of the size of the conidia is limited and measurements of more than 4 × 30 conidia will add no further information.

Growth habits, colour [10] and the cardinal temperatures (minimum, optimum, maximum) were taken from cultures grown on PDA in the laboratory or in the dark, respectively.

## Results

The differentiation of the *F. sambucinum* s. 1. strains into three groups proved to be correct. Of the originally 140 strains 53% belonged to group I, 20% to group II and 19% to group III. Therefore three species: *F. sambucinum* s. str., *F. torulosum comb. nov.*, and *F. venenatum* sp. nov. which correspond to the groups respectively are described and delimited.

***F. sambucinum* Fuckel s. str.** – Symb. Mycol. 1869; 167

= *F. roseum* Link (nomen ambiguum) – Diss. I. – Mag Ges naturf Freunde, Berl 1809; 7: 25–45.

= *F. sulphureum* Schlecht. sensu Wollenw. – Flora Berol 1824; 139.

= *F. trichoteciodes* Wollenw. – in Jamieson & Wollenweber, J Wash Acad Sci 1912; 2: 146–152.

= *F. sambucinum* Fuckel f. 2 Wollenw. – Z Parasitenk 1931; 3: 357.

= *F. sambucinum* Fuckel f. 6 Wollenw. – Z Parasitenk 1931; 3: 358.

= *F. sambucinum* Fuckel var. *minus* Wollenw. – Z Parasitenk 1931; 3: 358.

= *F. roseum* Link ex Gray emend. Snyder & Hansen pr. p. – Am J Bot 1945; 32: 663

Teleomorph: *Gibberella pulicaris* (Fr. ex Fr.) Sacc. – Michelia 1877; 1: 43

*Table 2.* Test strains used in the European *Fusarium sambucinum* Project

| Collection number | Species | Colours of culture on wortager | Matrix | Country of origin | Collector or depositor |
|---|---|---|---|---|---|
| Group I | | | | | |
| 64 995 | *F. sambucinum* | yellowish | *Brassica oleracea* | Netherlands | Noordeloos |
| 64 678 | *F. sambucinum* | yellowish | *Triticum aestivum* | Switzerland | Thrane |
| 62 433 | *F. sambucinum* | yellowish | *Beta vulgaris* | Spain | Gerlach |
| 64 960 | *F. sambucinum* | yellowish | soil | Denmark | Thrane |
| 64 747 | *F. sambucinum* | rose | *Solanum tuberosum* | Fed. Rep. Germany | Langerfeld |
| 65 009 | *F. sambucinum* | rose | *Solanum tuberosum* | Italy (?) | Logrieco |
| 62 434 | *F. sambucinum* | rose | *Solanum tuberosum* | Iran | Gerlach |
| 64 226 | *F. sambucinum* | rose | *Solanum tuberosum* | Great Britain | Nirenberg |
| 64 998 | *F. sambucinum* | rose | *Solanum tuberosum* | France | Tivoli |
| 64 996 | *F. sambucinum* | brownish | *Solanum tuberosum* | France | Tivoli |
| 62 397 | *F. sambucinum* | red | *Solanum tuberosum* | Fed. Rep. Germany | Gerlach |
| 64 484 | *F. sambucinum* | red | *Solanum tuberosum* | Finland | Seppänen |
| 64 262 | *F. sambucinum* | red | *Glycine max* | Brazil | Nirenberg |
| 64 480 | *F. sambucinum* | red | *Solanum tuberosum* | Finland | Seppänen |
| Group II | | | | | |
| 64 990 | *F. torulosum* | red to brown | *Buxus sempervirens* | Netherlands | Noordeloos |
| 64 948 | *F. torulosum* | brownish | unknown | Denmark | Thrane |
| 64 479 | *F. torulosum* | brownish | *Solanum tuberosum* | Finland | Seppänen |
| 64 645 | *F. torulosum* | whitish | *Solanum tuberosum* | Denmark | Thrane |
| 64 988 | *F. torulosum* | red | *Hordeum vulgare* | Hungary | Széczi |
| 62 398 | *F. torulosum* | red | *Betula verrucosa* | Fed. Rep. Germany | Gerlach |
| 63 933 | *F. torulosum* | red | *Triticum aestivum* | Australia | Gerlach |
| 64 465 | *F. torulosum* | beige | *Triticum aestivum* | Fed. Rep. Germany | Nirenberg |
| Group III | | | | | |
| 64 935 | *F. venenatum* | reddish | *Solanum tuberosum* | Poland | Latus |
| 65 030 | *F. venenatum* | reddish | *Zea mays* | Fed. Rep. Germany | Nirenberg |
| 64 757 | *F. venenatum* | reddish | *Humulus lupulus* | Fed. Rep. Germany | Gerlach |
| 64 537 | *F. venenatum* | reddish | *Triticum aestivum* | Austria | Nirenberg |
| 64 478 | *F. venenatum* | brownish | *Solanum tuberosum* | Finland | Seppänen |
| 65 031 | *F. venenatum* | beige | *Zea mays* | German Dem. Rep. | Nirenberg |
| Group IV, strains resembling *F. sambucinum* s. l. morphologically | | | | | |
| 64 371 | *F* sp. | yellowish | *Curcurbita maxima* | New Zealand | Laundon |
| 64 280 | *F.* cf. *sambucinum* | brownish | *Ruschia comosa* | Fed. Rep. Germany | Reinecke |
| 64 993 | *F. sambucinum* | brownish | unknown | Netherlands | Hermanides-Nijhof |
| 63 575 | *F.* sp. | yellowish | *Medicago sativa* | New Zealand | Gerlach |
| 64 351 | *F. semitectum* var. *semitectum* | beige | *Helianthus annuus* | Jugoslavia | Nirenberg |

Type: Herbarium Fuckel, 1894, No. 595, in herbarium Barbey-Bossier (G), Fungi rhenani 241 (Fig. 4).

Cardinal temperatures: 2.0, 25.0, 32.5 °C.

Colonies: Growing in concentric rings, fast, reaching 5.0 cm in diam. in five days at 20 °C in the dark on PDA, margins lobed (Fig. 1), degenerated strains growing somewhat faster, with margins not lobed at all (Fig. 1).

Colony reverse: Yellowish white, brownish orange, greyish orange, ruby with brown dots. Aerial mycelium: Generally abundant, floccose to felt-like, white, yellowish white, to greyish orange.

*Table 2.* Continued

| Collection number | Species | Colours of culture on wortager | Matrix | Country of origin | Collector or depositor |
|---|---|---|---|---|---|
| Group V | | | | | |
| 63 602 | *F. bactridioides* | red | *Cronartium ribicola* | USA (Arizona) | Wollenweber |
| Group VI | | | | | |
| 63 714 | *F. sarcochroum* | beige | *Viscum album* | Switzerland | Gerlach |
| Group VII | | | | | |
| 64 918 | *G. pulicaris* | rose | palm tree | Indonesia | Samuels |
| 62 719 | *G. pulicaris* | yellowish-rose | *Pterocarya fraxinifolia* | Iran | Gerlach |
| Group VIII, strains functioning as controls | | | | | |
| 64 218 | *F. culmorum* | reddish | *Hordeum vulgare* | Finland | Nirenberg |
| 64 545 | *F. cerealis* | reddish | *Triticum durum* | Fed. Rep. Germany | Nirenberg |
| 64 640 | *F. compactum* | red | herbs | France | Thrane |
| 64 225 | *F. lateritum* | rose | *Malus domestica* | Fed. Rep. Germany | Nirenberg |

*Fig. 1.* Reverse of *F. sambucinum* s.l. cultures on PDA after 9 days growing in the lab.: Clockwise from the upper left: *F. sambucinum* s. str. (BBA 64 995), *F. sambucinum* s. str., degenerated culture variant (BBA 64 993), *F. torulosum* (BBA 65 417) and *F. venenatum* (BBA 65 416).

Sclerotia: abundant in fresh isolates, of brownish orange to brown colour, shape cauliflower-like.

Sporulation: starting in the aerial mycelium, later on sclerotial stroma as sporodochia, tinged greyish orange, brownish orange, brown, and ruby, depending on colour of the isolate.

Odour: In some strains faintly mushroom-like.

Conidiophores: At first arising as single lateral phialides, later in sporodochia or pionnotes, consisting of delicate, long multicelled stalks, that branch vigorously, terminating in phialides.

Phialides: Monophialidic, cylindrical to doliiform, often with a collarette, mostly 14.0–18.0 × 3.8–4.5 $\mu$m.

Conidia: On SNA rather uniform in size, compact, falcate, with a constricted apical cell and a distinct pedicellate basal cell, mostly 3- to 5-septate, in DL the 3-septate conidia prevailed (Fig. 2), in D the 5-septate ones did (Fig. 3), they measured:

3-septate DL (28.4-)30.6-37.2(-40.0) × (4.0-)4.2-5.2(-6.0) $\mu$m
3-septate D (24.0-)24.1-26.9(-28.0) × (4.0-)4.1-4.7(-5.0) $\mu$m
5-septate DL (34.0-)36.8-44.4(-44.8) × (4.4-)4.5-5.3(-5.5) $\mu$m
5-septate D (26.8-)29.1-32.9(-35.2) × (4.2-)4.6-5.4(-5.6) $\mu$m

Chlamydospores: Not found within 14 days growing on SNA in the dark.

Occurence: *Beta vulgaris*, *Brassica oleracea*, *Glycine max*, *Pterocarya fraxinifolia*, *Solanum tuberosum*, *Triticum aestivum*, *Vicia faba*, soil.

Delimitation: *F. sambucinum* is a fast growing species with ruby, greyish orange or brownish orange sub-

strate mycelium, width of sporodochial conidia on SNA in DL 4.9 µm, in D 5.0 µm, no chlamydospores are found within 14 days on SNA in D, growing margin weakly lobed or not at all; its morphology is unique and can not be confused with any other *Fusarium* species (see Table 3).

***F. torulosum* (Berk. et Curt.) Nirenberg**

≡ *Fusidium torulosum* Berk. et Curt. – North Am Fungi, No. 679.

= *Fusoma torulosum* (Berk. et Curt.) Sacc. – Syll Fung 1886; 4: 220.

= *F. sclerodermatis* Oudem. – Nederl Kruidk Arch II 1889; 5: 516.

= *F. polymorphum* Matruchot – Rech Dével Mucéd 1892: 84–91 Table 7, Fig. 6-14.

= *F. subpallidum* Sherb. – Mem Cornell Univ Agric Exp Stn 1915; 6: 175.

= *F. sambucinum* Fuckel var. *coeruleum* Wollenw. – Ann Mycol 1917; 15: 55.

= *F. elegantum* Pratt – J Agr Res 1918; 8: 84.

= *F. sambucinum* Fuckel f. 1 Wollenw. – Z Parasitenk 1931; 3: 356.

= *F. sambucinum* f. 5 Wollenw. – Z Parasitenk 1931; 3: 358.

= *F. roseum* Link ex Gray emend. Snyder & Hansen pr. p. – Am J Bot 1945; 32: 663.

= *F. pezizoideum* (Berk. et Curt.) Sacc. – Syll Fung 1886; 4: 711.

= *F. rostratum* Speg. – in Wollenweber & Reinking, Die Fusarien, Berlin: Verlag Paul Parey, 1935: 76.

Teleomorph: ?*Gibberella pulicaris* (Fr.) Sacc. var. *minor* Wollenw. – Z Parasitenk 1931; 3: 356.

Cardinal temperatures: 2.0, 27.5, 37.5 °C.

Colonies: Growing in narrow concentric rings, slowly, reaching 2.0–2.5 cm in diam. in 5 days on PDA in the dark at 20 °C, margins lobed (Fig. 1).

Colony reverse: Ruby, with growing margin white, degenerated cultures may be yellowish white or brown.

Aerial mycelium: Short, dense, lannose to felt-like, orange white, shining greyish rose above the ruby mycelium of the substrate; growing margin 0.5–1.0 cm, pure white.

Sclerotia: Present bluish black, cauliflower-like in shape.

Sporulation: Starting comparatively late, usually in orange sporodochia.

Odour: Not perceptible.

Conidiophores: At first arising as single lateral phialides on the hyphae, later in sporodochia branching densely.

Phialides: Monophialidic, cylindrical to slightly doliiform, measuring 14.0–18.0 × 3.2–4.2 µm.

Conidia: On SNA from sporodochia, in DL slender, falcate, with a pointed apical cell and a distinct pedicillate basal cell (Fig. 5), in D relatively compact with a rounded apical cell and a truncate basal cell, mostly 5-septate (Fig. 6), measuring:

5-septate DL (32.0-)35.8-40.0(-42.0) × 4.0-4.4 µm

5-septate D (28.8-)31.7-35.9(-37.0) × (4.4-)4.6-5.4(-5.6) µm.

Those in D resemble the conidia of the type specimen, *F. bactridioides* (Fig. 9). Often 0- to 1-septate conidia occur in DL and D (Fig. 7).

Chlamydospores: Formed in the dark on SNA usually in long chains or/and clusters (Fig. 8).

Occurence: *Beta vulgaris*, *Betula verrucosa*, *Buxus sempervirens*, *Hordeum vulgare*, *Pinus sylvestris*, *Solanum tuberosum*, *Triticum aestivum*, soil.

Delimitation: *F. torulosum* is a slow growing species with ruby substrate mycelium and a white, lobed growing margin, width of sporodochial conidia on SNA in DL 4.2 µm, in D 4.8 µm, often 0- and 1-septate microconidia are formed, chlamydospores are produced within 14 days on SNA in D, growth habit on wort agar similar to cultures of *F. lateritium* or *F. flocciferum* Corda, not considering colour (see Table 3).

***F. venenatum* sp. nov.**

= *F. sambucinum* Fuckel var. *coeruleum* Wollenw. sensu Booth – The genus Fusarium 1971: 171–172.

= *F. sambucinum* Fuckel var. *coeruleum* Wollenw. sensu Gerlach & Nirenberg – Mitt Biol Bundesanst Land- Forstw 1982; 209: 213–216.

= ? *F. sulphureum* Schlecht. – Flora Berol 1824; 139.

= ? *F. culmorum* W.G. Smith var. *cerealis* (Cooke) Wollenw. pr. p. – Z Parasitenk 1931; 3: 362.

= ? *F. equiseti* (Corda) Sacc. var. *crassum* Wollenw. – Z Parasitenk 1931; 3: 333.

= *F. roseum* Link. emend. Snyder & Hansen pr. p. – Am J Bot 1945; 32: 663.

Coloniae in agaro PD 20 °C in 5 diebus circiter 44 mm diametro, candidae usquae pallide-roseae plerumque rubrae centro flavae, lannosae; reverso rubrae, rario

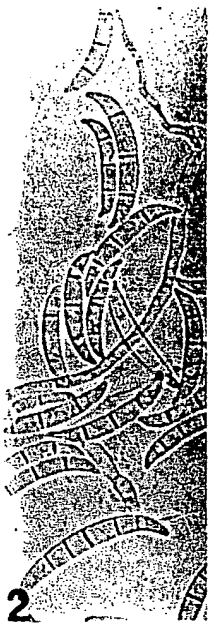



*Fig. 2. F. sambucinu*
conidia on SNA in dar

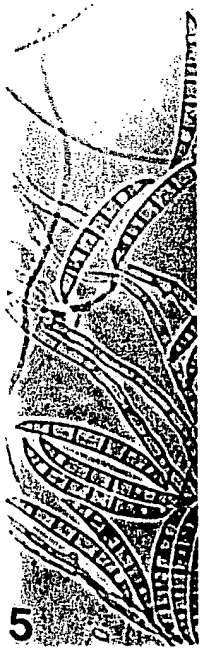



*Fig. 5. F. torulosum*;
on SNA in darkness a

ıg as single lateral
ı sporodochia branch-

ırical to slightly doli-
3.2–4.2 μm.
ɔchia, in DL slender,
l cell and a distinct
in D relatively com-
l and a truncate basal
measuring:

40.0(-42.0) ×

35.9(-37.0) ×
4(-5.6) μm.

idia of the type spec-
9). Often 0- to 1-
d D (Fig. 7).
dark on SNA usually
Fig. 8).
*ıla verrucosa*, *Buxus*
*are*, *Pinus sylvestris*,
*aestivum*, soil.
slow growing species
ı and a white, lobed
sporodochial conidia
D 4.8 μm, often 0-
e formed, chlamydo-
4 days on SNA in D,
similar to cultures
*n* Corda, not consider-

*coeruleum* Wollenw.
Fusarium 1971: 171–

*coeruleum* Wollenw.
Mitt Biol Bundesanst
3–216.
Flora Berol 1824; 139.
var. *cerealis* (Cooke)
ık 1931; 3: 362.
ar. *crassum* Wollenw.

der & Hansen pr. p. –

diebus circiter 44 mm
ide-roseae plerumque
reverso rubrae, rario

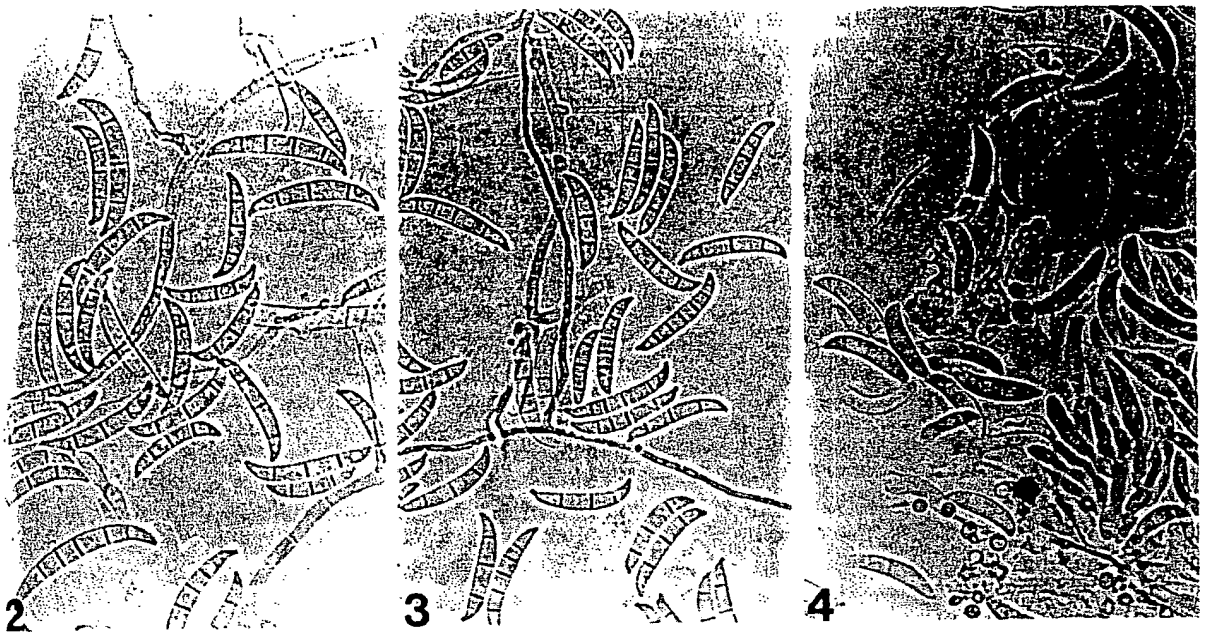



*Fig. 2.* *F. sambucinum*; sporodochial conidia on SNA under continuous black light at 20 °C; × 500. *Fig. 3.* *F. sambucinum*; sporodochial conidia on SNA in darkness at 20 °C; × 500. *Fig. 4.* *F. sambucinum*, type (G); sporodochial conidia from *Sambucus nigra*; × 500.

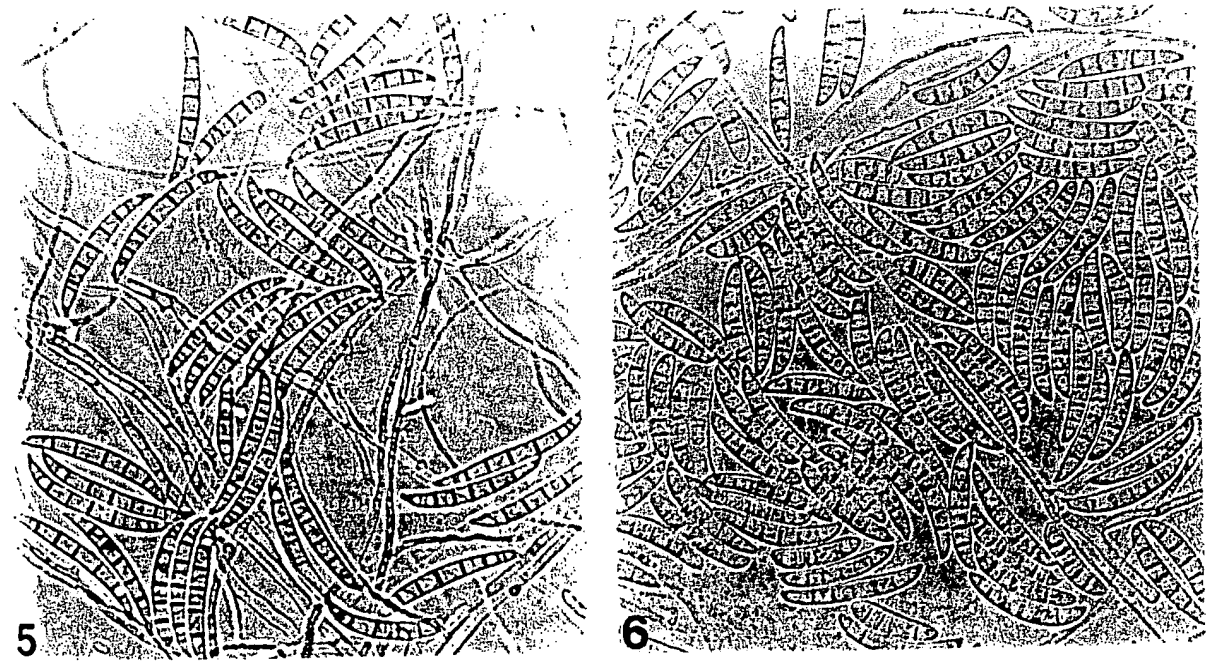
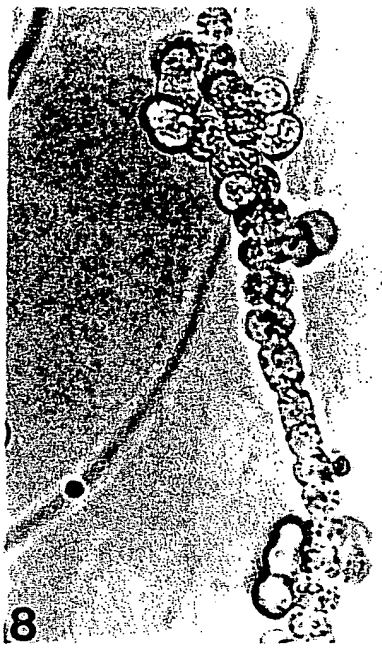



*Fig. 5.* *F. torulosum*; sporodochial conidia on SNA under continuous black light at 20 °C; × 500. *Fig. 6.* *F. torulosum*; sporodochial conidia on SNA in darkness at 20 °C; × 500.



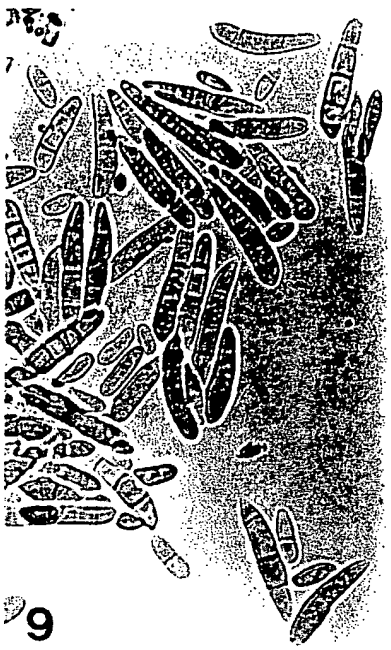


*Fig. 7.* *F. torulosum*; sporodochial conidia and microconidia under continuous black light at 20 °C; × 500. *Fig. 8.* *F. torulosum*; chains of chlamydospores on SNA in darkness at 20 °C, × 500. *Fig. 9.* *F. bactridioides*, type (NY); sporodochial conidia from *Cronartium ribicola*; × 500.

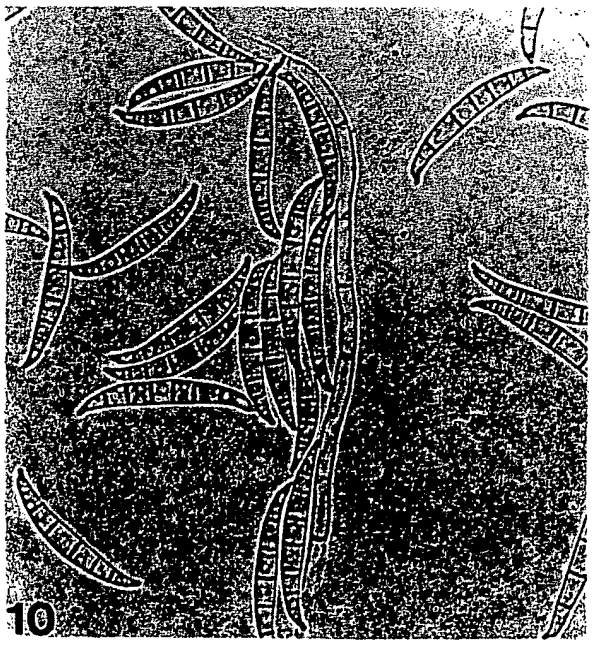


*Fig. 10.* *F. venenatum*; sporodochial conidia on SNA under continuous black light at 20 °C; × 500. *Fig. 11.* *F. venenatum*; sporodochial conidia and chlamydospores on SNA in darkness at 20 °C; × 500.

*Fig. 12.* *F. venenatum*; c
potato tuber; × 500.

*Table 3.* Morpl

| Species | |
|---|---|
| *F. sambucinu* | |
| s. str. | |
| *F. torulosum* | |
| *F. venenatun* | |

cremeae. Tempera
Conidia in mycelic
lata in sporodochii
na, falciformia, ap
5-septata, in agerc
(-42.4) × (4.8-)5.
36.4-40.0(-42.0) ×
phora simplicia v

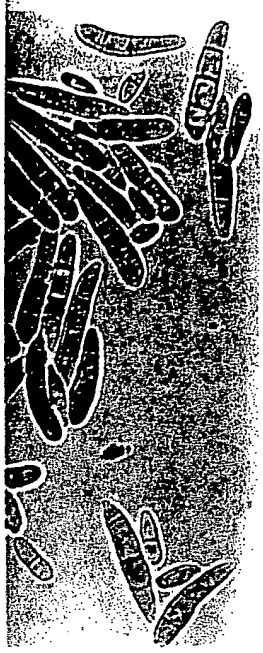
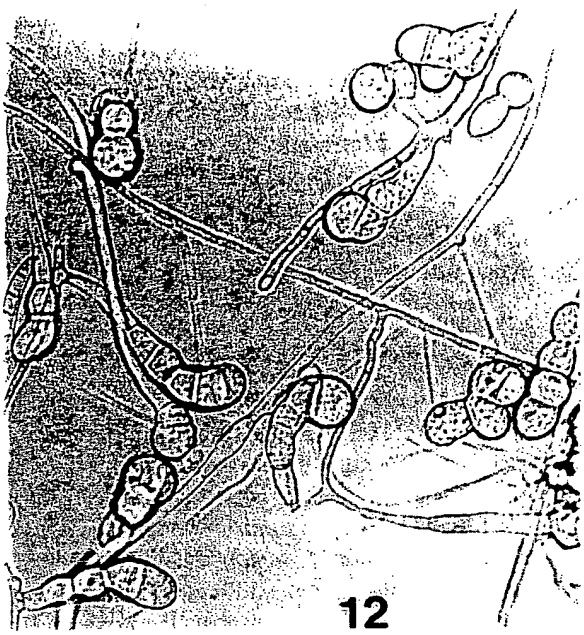
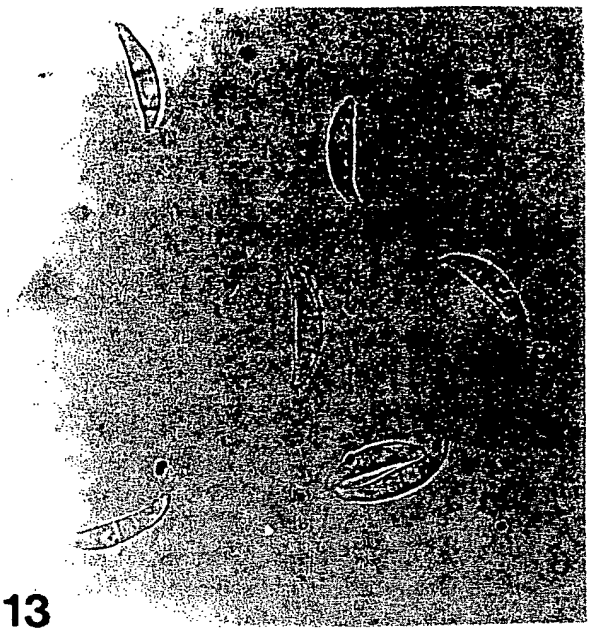


Fig. 8. *F. torulosum*; chains of a from *Cronartium ribicola*; ×



12



13

*Fig. 12. F. venenatum*; chlamydospores on SNA in darkness at 20 °C; × 500. *Fig. 13. F. sulphureum*, type (HAL); sporodochial conidia from potato tuber; × 500.

A 11

*. F. venenatum*; sporodochial

*Table 3.* Morphological and physiological differences within *Fusarium sambucinum* s. l.

| Species | Growth at 20 °C on PDA (mm/day) | Cardinal temperatures (°C) | Micro-conidia | Size of 5-septate conidia on SNA $\bar{x}$ | Size of 5-septate conidia on SNA in DL (μm) $\bar{x}$ | Chlamydospores in D (μm) |
|---|---|---|---|---|---|---|
| *F. sambucinum* s. str. | 5.0 | 2.0, 25.0, 32.5 | (−) | 40.6 × 4.9 | 31.0 × 5.0 | – |
| *F. torulosum* | 2.2 | 2.0, 27.5, 37.5 | + | 37.9 × 4.2 | 33.8 × 4.8 | Intercalary, in long chains |
| *F. venenatum* | 4.4 | 2.0, 25.0, 35.0 | – | 38.6 × 5.5 | 38.4 × 6.3 | Laterally, in short, hooked chains |

cremeae. Temperationes cardinales 2.0, 25, 35 °C. Conidia in mycelio aerio nulla, conidia etiam accumulata in sporodochiis brunneis vel bruneo-rubris, hyalina, falciformia, apice acerosa, pedicellata, plerumque 5-septata, in agero SN et luce (DL) (35.4-)37.0-40.2 (-42.4) × (4.8-)5.1-5.9(-6.2) μm, obscure (D) (34.2-)36.4-40.0(-42.0) × (5.2-)5.7-6.9(-7.4) μm. Conidiophora simplicia vel ramosa cum phialidibus simplicibus, doliiformibus. Sclerotia et odor absunt. Chlamydosporae singulae in hyphis, saepe lateraliter catenis brevibus curvatis, griseae.

Habitat: in culmis Tritici aestivi.

Aera geographica: Austria.

Holotypus: in culto agaro desiccatus Fusarii venenati Nirenberg(CBS 458.93).

Exholotypus: BBA 64 537, CBS 458.93.

Cardinal temperatures: 2.0, 25.0, 35.0 °C.

Colonies: Growing in concentric rings, reaching 4.3–4.6 cm in diam. in 5 days on PDA in the dark at 20 °C, margins lobed (Fig. 1).

Colony reverse: Ruby to reddish brown, seldom greyish orange, degenerated cultures often brown.

Aerial mycelium: Long, dense, cottony white, orange grey, or ruby, in the middle of the colony sometimes brownish orange.

Sclerotia: Not found.

Sporulation: In brown to reddish brown sporodochia.

Odour: Not perceptible.

Conidiophores: At first arising as single lateral phialides on hyphae, soon branching generously, terminating in phialides.

Phialides: Monophialidic, mostly doliiform, measuring 12.0–20.0 × 4.8–5.0 μm.

Conidia: On SNA in DL relatively slender, falcate with a pointed apical cell and a pedicellate basal cell (Fig. 10), in D wider, resembling conidia of *F. cerealis*, *F. compactum* or *F. sulphureum* (Fig. 13), mostly 5-septate (Fig. 11), measuring:

5-septate DL (35.4-)37.0-40.2(-42.4) × (4.8-)5.1-5.9(-6.2) μm.
5-septate D (34.2-)36.4-40.4(-42.0) × (5.2-)5.7-6.9(-7.4) μm.

Chlamydospores: On SNA in D pale to light grey coloured, single or in lateral hooked, short chains. (Fig. 12).

Occurence: *Humulus lupulus*, *Solanum tuberosum*, *Spinacia oleracea*, *Triticum aestivum*, *Zea mays*, soil.

Delimitation: *F. venenatum* is a fast growing species with intenstive ruby substrate mycelium, lobed growing margin, width of sporodochial conidia on SNA in DL 5.5 μm, in D 6.3 μm, resembling those of *F. cerealis* and *F. compactum* in D or conidia of the type specimen of *F. sulphureum*, chlamydospores pale grey coloured, mostly in lateral somewhat hooked short chains (see Table 3).

Distribution: Europe.

Holotypus: Dried culture of *F. venenatum* Nirenberg (CBS 458.93).

Exholotypus: BBA 64 537, CBS 458.93.

Of group IV, only two isolates could be identified in the course of the project: BBA 64 993 as *F. sambucinum* and BBA 64 351 as *F. semitectum* Berk. et Rav. var. *semitectum*. The identity of the others in that group remain unsolved. *F. bactridioides* (group V) looks very similar to *F. torulosum* (Fig. 6, 9), but since the only existing culture is old and degenerated, a questionmark has to remain. The one strain (BBA 63 714) of *F. sarcochroum* (group VI) does not look like one of the three described species and has to be accepted as a species of its own. The two isolates originating from *G. pulicaris* (group VII) are designated as *F. sambucinum* s. str. The morphology of the four control strains did not match with any other strain used in this project.

## Discussion

Since the three taxa exhibited unique morphological features, and no strong affinities between them could be found otherwise, all of them were given species rank: *F. sambucinum* s. str., *F. torulosum* and *F. venenatum*. Degenerated cultures of *F. sambucinum* s. str. and of *F. torulosum*, however, become difficult to identify, since both can produce slender sporodochial conidia and 0- to 1-septate microconidia (= sign of degeneration in *F. sambucinum* s. str.). BBA 64 993 represents such a culture (Fig. 1).

It could be demonstrated that *F. sulphureum* is not a synonym for *F. sambucinum* s. str. as Wollenweber & Reinking [6] thought. *F. sulphureum* might be synonymous with either *F. cerealis* or *F. venenatum*. Only rDNA sequencing or RAPD PCR of the type material will resolve this question. Since *F. sulphureum* was used for such a long time in a different sense, it will probably become a nomen dubium such as *F. roseum*.

The question as to whether *F. bactridioides* and/or *F. sarcochroum* are synonyms of *F. sambucinum* could not be solved by their morphological characteristics. The answers might be found by investigations in which methods of molecular biology are employed.

O'Donnell [11] sequenced the ITS region of *F. sambucinum* rDNA and found three types (A, B, C) with quite high divergence between them. Type B corresponds with the *F. sambucinum* s. str. strains. If they belong to one mating-population, as he stated, it has to be assumed, that types A and C are different, non-European populations within the species *F. sambucinum* s. str.

**Acknowledgement**

The author thanks M
skillful technical as

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## Acknowledgements

The author thanks Mrs Heidrun Anders for her reliable, skillful technical assistance.

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